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16. ABSTRACT

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Toxicological Comparison of Natural and Cultured Populations of *Acartia tonsa* to Cadmium, Copper, and Mercury

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variability of cultured populations was less than that of field populations. The 96-h LC50 concentrations for cultured (F_1) populations were 90 μg Cd/L, 31 μg Cu/L, and 10 μg Hg/L. The presence and abundance of ova and unicellular glands in the circumesophogeal region were used as criteria for evaluating the nutritional condition of test populations.

Key words: marine, metals, bioassay, culture, calanoid copepods, toxicity, histology, filial generations

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Acartia tonsa en culture a une réaction toxicologique reproductible durant six générations. Il n'y a pas de différences statistiquement significatives dans les réactions des générations F_1 et F_6 au cadmium, au cuivre et au mercure. La façon dont les populations cultivées et sauvages (parentales) exposées au cadmium réagissent n'est pas statistiquement différente. La variabilité des réponses des populations cultivées est moindre que celle des populations sauvages. Les CL-50 après 96 h pour les populations cultivées (F_1) sont de 90 μg Cd/L; 31 μg Cu/L; et 10 μg Hg/L. Comme critères d'évaluation de la condition alimentaire des populations d'essais, nous avons utilisé la présence et l'abondance des oeufs et les glandes unicellulaires dans la région circumoesophagienne.

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HISTORICALLY, marine bioassay organisms were collected or spawned from field populations. This imposes restraints upon the researcher in terms of continuous availability of assay organisms in large quantities, nutritional and genetic uniformity, variability in age of the organisms, and incidence of disease. These restraints are particularly important in programs where continuous or repetitive testing is required, or a comparative data base is being compiled.

Static and continuous flow systems have been used successfully to produce successive generations of calanoid copepods for toxicological studies (Gentile et al. 1974). These meet criteria necessary for all organisms used for toxicological purposes. First, and most importantly, cultured species must have a nutritional history that is both qualitatively and quantitatively similar to those occurring in their natural environment so that their response will not be biased by nutritional condition. Second, species cultured for several generations must have reproducible responses to stress; and third, they must manifest a sensitivity similar to field populations.

The objectives of this study are (1) to examine the response reproducibility and sensitivity of the calanoid copepod *Acartia tonsa* to three metal pollutants, (2) to compare responses of cultured and field populations, and (3) to employ histological techniques for the assessment of the nutritional state of test organisms. Implicit in this approach is that both response reproducibility and sensitivity reflect the nutritional condition of the organisms.

Materials and methods — Test organisms (*Acartia tonsa*) were collected August 13, 1973, from a depth of 1 m with a 0.5-m (no. 10 mesh; 150 μM) plankton net in the Pettaquamscutt River estuary, Narragansett, R.I. Three- to 5-min plankton tows were taken from the Bridgetown Bridge. Current speed was approximately 0.5 m/s. Ambient water temperature and salinity were 24°C and 10 ‰, respectively. Tow material was diluted fivefold with river water in insulated containers and returned to the laboratory within 30 min. Five, 200-mL samples of tow material were transferred to 190 × 100-mm Pyrex crystalizing dishes and diluted to ~2000 mL with filtered (0.45 μM) river water. These samples were incubated overnight at 20°C and 1000 lx on a 14L:10D cycle. Adult female *A. tonsa* were then sorted using standard taxonomic criteria (Wilson 1932) and placed in generation cages. After 24 h, eggs were collected and used to start two mass cultures. The details of the culture procedure are given in Gentile et al. (1974).

Acartia tonsa feeds in the water column; therefore, food organisms were maintained in the water column by stirring at ≤25 rpm. Previous culture systems for *A. tonsa* failed to keep food available (Zillioux and Lackie 1970; Heinle 1969). A second consideration was the quality and quantity of food organisms. *Acartia tonsa* cultures were often fed unrealistic algal food densities poor in the naturally occurring food organisms, planktonic diatoms, which are more difficult to culture (Zillioux and Wilson 1966; Wilson and Parrish 1971; Heinle 1969). The cultured filial generations in this study were fed four algal species. *Isochrysis galbaena* (5.0×10^6/L) and *Rhodomonas baltica* (3.0×10^6/L) have been used historically for culturing copepods. *Skeletonema costatum* (5.0×10^6/L) and *Thalassiosira pseudonana* (7.0×10^6/L) provide the natural staple diet for the genus *Acartia*. *Skeletonema costatum* (6–10 μM) is of a size range preferred by adults, while *T. pseudonana* (2–4 μM) is suitable for naupliar stages. The total algal cell density (2.0×10^7/L) reflects the maximum algal cell density in Narragansett Bay (Smayda 1957, 1973; Pratt 1959).

Additional collections of *A. tonsa* were made as described above from lower Narragansett Bay. *Acartia tonsa* were held at 20°C and 30 ‰ for 24 h and fed (as above) before testing.

Histological examinations were made on all collected and cultured populations according to methods of Barszcz and Yevich (1976).

TABLE 1. Analysis of cadmium toxicity (LC50 in μg Cd^{+2}/L) to the calanoid copepod *Acartia tonsa* after 96-h exposure.

Probit analysis	P[a]	F_1[b]	F_6[c]	B[d]
Mean LC50	337	90	220	122
Upper limit (95%)	530	135	313	341
Lower limit (95%)	184	58	138	60
Analysis of variance				
Source	df	SS	MS	F
Total	74	96 029		
Concentration	4	75 850	18 962	106.9[e]
Population	4	3 801	950	5.3[e]
Error	50	8 862	117	

[a]Parental stock; [b]first filial generation; [c]sixth filial generation; [d]bay population; [e]F .95(4, 50) = 2.56.

TABLE 2. Analysis of copper toxicity (LC50 in μg Cu^{++}/L) to the calanoid copepod *A. tonsa* after 96-h exposure.

Probit analysis	F_1[a]	F_6[b]	B[c]	
Mean LC50	31	55	17	
Upper limit (95%)	44	76	29	
Lower limit (95%)	21	36	9	
Analysis of variance				
Source	df	SS	MS	F
Total	89	105 264		
Concentration	4	80 872	20 218	131.5[d]
Population	3	7 316	1 463	9.5[e]
Error	60	9 225	154	

[a]First filial generation; [b]sixth filial generation; [c]bay population; [d]F .95(60) = 2.52; [e]F .95(3, 60) = 2.80.

Static bioassays were performed on selected filial generations from cultures (F_1, F_2, F_6), on the parental *A. tonsa* field population from Pettaquamscutt River (P), and on field populations of *A. tonsa* (B) collected from Narragansett Bay. Forty-five organisms, as three replicates of 15, were exposed in each of five toxicant concentrations and a control. Duration of the experiment was 96 h. Dead animals were removed and recorded daily. Exposure conditions were 20°C and 10‰ for the P and F generations of *A. tonsa*; 20°C and 30‰ for B populations. Salinity differences reflect those found in collection sites. Light was programmed for 14L:10D for all *A. tonsa* studies to coincide with conditions in the field at the time of collection.

Stock solutions of cadmium, copper, and mercury were prepared by dilution atomic absorption primary standards (Alpha Analytical Lab., N.J.) in deionized water (18 Mohm at about 20°C). Dosages are reported as nominal values.

To obtain the toxicant LC50 and the upper and lower 95% confidence interval (Finney 1971), mortality response patterns were first analyzed by probits. The mortality response patterns of the various source populations were also analyzed by two-way analysis of variance. When necessary, the treatments' sums of squares were partitioned to identify which populations were different (Snedecor and Cochran 1967).

Results and discussion — Studies using cadmium were the most pertinent because, in this series, comparison testing with the parental (field) population was possible. The bioassay data for 96-h exposure were tested with analysis of variance (Table 1) to determine whether the toxicological responses observed for each source population to cadmium were the same. There were significant ($P < .05$) differences in responses due to population source and due to toxicant concentration. There were no differences ($P \geq .05$) between the P, F_1, and F_6 populations, or between P, F_1, and B populations, but significant differences ($P < .05$) occurred between the F_6 and B populations.

Graphical estimation of the F_2, 96-h LC50 resulted in a value of 190 μg Cd^{+2}/L which is within the range of both F_1 and F_6 generations and bay (B) data. The difference between the upper confidence interval and the mean LC50, divided by the mean LC50, produces a simple index of response variability within the assay population. These values are $P = 57\%$; $F_1 = 50\%$; $F_6 = 42\%$; $B = 179\%$; which indicate that the B population has a greater intrinsic variability than the cultured or parental populations.

TABLE 3. Comparison of LC50 values (μg Hg^{+2}/L) for filial generations of *A. tonsa* exposed to mercury.

Organism (population)	Exposure time (h)			
	24	48	72	96
F_1	17.5	17	13	10
F_2	20	19	17	14
F_6	22	19	16	15

Acartia tonsa F_1, F_2, F_6, and B populations were exposed to copper. Analysis of variance of toxicity data to F_1, F_6, and B populations indicates significant ($P < 0.05$) differences in their responses (Table 2). Filial generations F_1 and F_6 were not statistically different from each other. However, the *A. tonsa* B population was significantly different from filial populations. The F_2 population had a 96-h LC50 of 64 μg/L. Using the response variability index described previously, we found that the cultured populations ($F_1 = 38.5\%$, $F_6 = 38\%$) had less variability than the bay population ($B = 71\%$).

Data for mercury (Table 3) was not amenable to probit analysis because the response patterns were not suitably graded over time. That is, mercury toxicity had reached its maximum effect within 24 h after exposure was initiated. Since exposures were static, it is possible that mercury was no longer available in a biologically active form.

The toxicological data for cadmium and copper indicate that there is no statistically significant difference in cultured populations after six filial generations. Though not analyzed statistically, the mercury data from filial

generation bioassays show very little response variation. Comparison of field and cultured populations did, however, reveal inconsistencies.

The agreement between the parental (field) and successive filial generations exposed to cadmium suggests that these populations were nutritionally similar at the time of testing. In both cadmium and copper studies, the only statistically significant differences between source populations involved the *A. tonsa* bay populations. This could be the result of salinity differences in the test conditions, the lack of acclimation with feeding before testing, and the high response variability characteristic of the bay populations. The single point sample represented by our field populations reflects only one set of nutritional and environmental conditions in a constantly changing continuum. The culture conditions were chosen to represent optimal field conditions. Therefore, unless the tested field populations corresponded to these conditions, agreement would be unlikely. Ideally, bioassays should be conducted on a series of natural populations to account for changes in food (quality and quantity) and age structure of test populations. Such studies would describe the expected natural response variability with which one could confidently compare data derived from cultured populations.

Histological examinations were performed on *A. tonsa* populations used for toxicological studies. The filial generations (F_1, F_2, and F_6) examined had abundant, normally developed ova and many unicellular esophogeal glands. The field populations (P, B), however, had few ova and a paucity of unicellular glands. Wilson and Parrish (1971) reported that egg production in *A. tonsa* is directly related to algal food density and, by inference, to nutrition. Gonzalez et al. (1975) suggested that the abundance of unicellular glands in the circumesophogeal region is indicative of nutritionally sound organisms. This hypothesis is based on the observation that starved *A. tonsa* populations show a progressive diminution of both ova and unicellular esophogeal glands. The abundance of ova and unicellular glands in our cultured populations affirms that both the quantity and quality of the algal diet used in this study (Gentile et al. 1974) provided adequate nutrition for *A. tonsa*.

The general lack of both ova and unicellular glands in our field populations is not unexpected when one considers the dynamic nature of phytoplankton–zooplankton cycles. The histological data indicate the field populations were nutritionally marginal compared with cultured populations. This is reflected in the greater variability in responses to toxicants demonstrated by the field populations.

We conclude that the culture methods employed in this study provide a nutritionally and physiologically consistent research organism. This enhances the confidence in laboratory derived data for this species and its application to natural populations.

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